Proceedings of the Consensus Meetings From the International Retinoblastoma Staging Working Group on the Pathology Guidelines for the Examination of Enucleated Eyes and Evaluation of Prognostic Risk Factors in Retinoblastoma

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• Retinoblastoma is the most common intraocular malignant childhood tumor in need of prospective clinical trials to address important unanswered questions about biology, treatment, and prognostic factors. Currently, there is controversy about the definitions for choroidal invasion and an inconsistency in the handling of eyes with retinoblastoma. The International Retinoblastoma Staging Working Group (IRSWG) composed of 58 participants from 24 countries on 4 continents had a series of Internet meetings to discuss the staging and tissue handling guidelines to reach consensus for adequate processing, establishing definitions of histopathologic risk factors, and reporting of enucleated eyes with retinoblastoma to serve as the basis for clinical trials and studies to validate the proposed criteria. The meetings were facilitated by the International Outreach Program of the St. Jude Children's Research Hospital through Cure4Kids. The retinoblastoma guidelines from the Children's Oncology Group, the French Society for Pediatric Cancers, the Association of Directors of Anatomic and Surgical Pathology, and some published data were the basis for this consensus document. Discussions of the feasibility, practicality, and efficacy of the guidelines and criteria resulted in this report. The consensus definitions reached included definition of massive choroidal invasion stated as a maximum diameter of invasive tumor focus of 3 mm or more that may reach the scleral tissue. Focal choroidal invasion is defined as a tumor focus of less than 3 mm and not reaching the sclera. Optic nerve invasion is classified as prelaminar, laminar, retrolaminar, or tumor at surgical margin, and the measurement of the depth of invasion should also be recorded. These guidelines also address handling of the enucleated eye with retinoblastoma in an efficient, practical, and feasible manner for a meaningful diagnosis. The consensus criteria reached by the IRSWG should be validated through prospective clinical trials and studies.

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Retinoblastoma—the most frequent primary intraocular tumor in children—lacks validation of a number of prognostic factors and requires a consensus on criteria for processing and interpreting histopathologic features. This is unlike uveal melanoma—the most frequent primary intraocular tumor in adults—in which histopathologic and molecular prognostic factors have been identified and validated through large retrospective and prospective studies and by molecular genetic assays. The current era of utilization of chemoreduction and adjuvant chemotherapy for retinoblastoma has prompted the need to standardize criteria across institutions and countries. However, there is scant literature available with clinical relevance and with prospective studies validating the indications for different chemotherapeutic approaches, especially adjuvant therapy.[1–13] To address this problem, a group of experts from around the world formed by the International Retinoblastoma Staging Working Group (IRSWG),[6,8] composed of 58 participants from 24 countries on 4 continents, held regular Internet Web-based consensus conferences to agree upon the recommendations to initiate a systematic vali-

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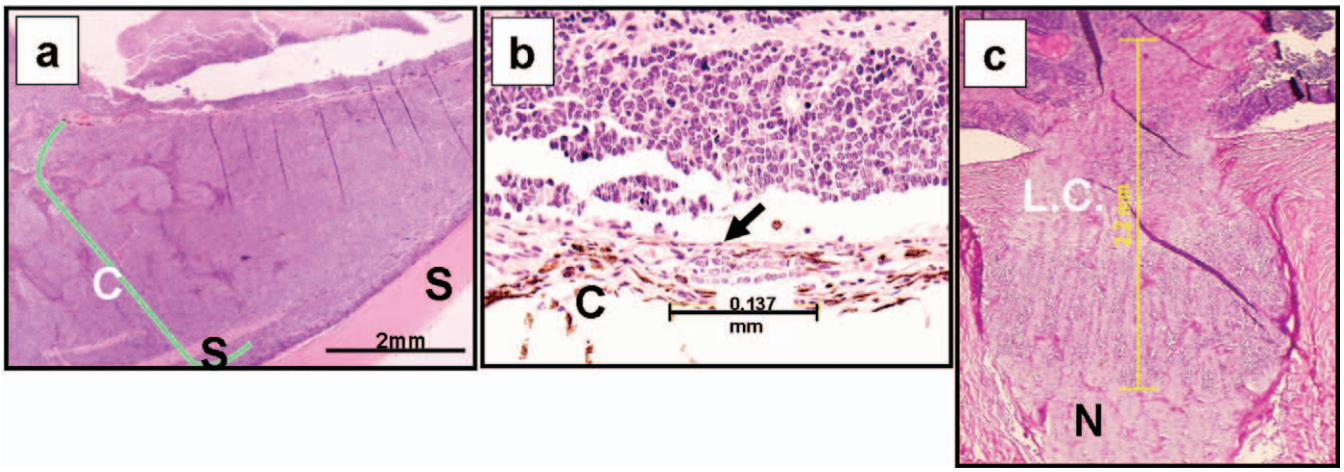

Figure 1. *a, Massive choroidal invasion is represented here by a large area of choroidal invasion (C) measuring more than 3 mm by tumor. The tumor reaches and replaces the inner layers of the sclera (S) (hematoxylin-eosin, original magnification ×2). b, Focal choroidal invasion is represented here by a focus of tumor in the choroid (C) that measures less than 3 mm (0.137 mm) in maximum diameter and does not reach the level of the sclera. The arrow points at Bruch membrane that separates choroid from retina (hematoxylin-eosin, original magnification ×20). c, Retinoblastoma tumor invading through the lamina cribrosa (L.C.) into the retrolaminar optic nerve (N). The maximum depth of invasion is 2.2 mm (Periodic acid–Schiff, original magnification ×4).*

dation of prognostic factors in eyes with retinoblastoma. The results of the consensus meetings are the subject of this communication and are the proposed basic processing techniques for obtaining best results in harvesting tissue for molecular studies and preservation of important histopathologic markers and for standardizing criteria for evaluation of high risk factors in enucleated eyes with retinoblastoma. The recommendations of the group, although representing a solid starting point, need to be prospectively validated through clinical trials and large studies.

CONSENSUS PROCESS

The meetings of the IRSWG were facilitated by the International Outreach Program of the St. Jude Children's Research Hospital in Memphis, Tennessee, through Cure4Kids. The retinoblastoma guidelines from the Children's Oncology Group, the French Society for Pediatric Cancers, the Association of Directors of Anatomic and Surgical Pathology, and the limited literature available related to the topic were the basis for this consensus document.[1–13] The discussions were focused on the feasibility, practicality, and efficacy of the proposed guidelines and on criteria based on the experience of the participants in different socioeconomic settings.

PROPOSED CONSENSUS CRITERIA

Consensus was reached for definitions of choroidal invasion. The proposed criteria to diagnose massive or significant choroidal invasion is when the maximum diameter (thickness or width) of invasive focus of tumor measures 3 mm or more in any diameter and, additionally, as a helpful landmark, when most of these tumors reach at least the inner fibers of the scleral tissue (Figure 1, a). The proposed criteria for focal choroidal invasion are defined as a tumor focus of less than 3 mm in any diameter (thickness or width) and not reaching the sclera (Figure 1, b). The group also discussed the importance of recognizing artifactual tumor seeding of the choroid and other extraocular tissues during fresh tumor retrieval to avoid overdiagnosing choroidal invasion. Artifactual seeding is composed of small groups of tumor cells, usually with many necrotic cells present inside natural spaces of the eye (eg, vascular choroidal and suprachoroidal space, anterior chamber, or subarachnoid space of the optic nerve), spaces created by sectioning artifacts, and/or on the surfaces of the eye (episclera, over meninges of optic nerve, and soft tissues attached to optic nerve). In contrast, true tumor invasion is composed of solid nests of tumor that have usually pushed or infiltrating borders, expanding and replacing the area of invasion. They typically lack necrosis, unless the tumor is extremely large.

The consensus for optic nerve invasion includes classification as prelaminar, laminar, retrolaminar, or tumor at surgical margin. The focus of tumor invasion should also be measured. To obtain the maximum depth of invasion into the optic nerve, the tumor should be measured from the level of the limiting membrane of the optic disc, the exit of the large central vessels, or, if none of these structures are preserved, from the level of the Bruch membrane to the deepest site of invasion (Figure 1, c).

The third part of the consensus was the agreement about the proposed guideline addressing processing of the enucleated eye with retinoblastoma to obtain best histopathologic results and fresh tumor for molecular testing. Two basic techniques were proposed to safely retrieve tumor without extensive contamination of ocular structures by tumor. Basically, the enucleated eye should be open immediately after the surgery to avoid denaturation of nucleic acids and proteins. The optic nerve should be measured for length and the cut margin should be obtained before opening the eye. The first technique proposed is the opening of a window in the sclera at the edge of the area containing most of the tumor. The window may be obtained using a trephine (corneal [for grafts]) or by using a sharp blade (Figure 2, a through c). Fresh tumor then should be retrieved from areas without necrosis. The other technique proposed is the aspiration of tumor by using a large bore needle by the introduction (under sterile conditions) of a 22-gauge needle through the sclera posteriorly to the lens according to a slightly oblique anteroposterior course under visual control (Figure 2, d). When the needle is situated in the tumor, tumor material is aspirated

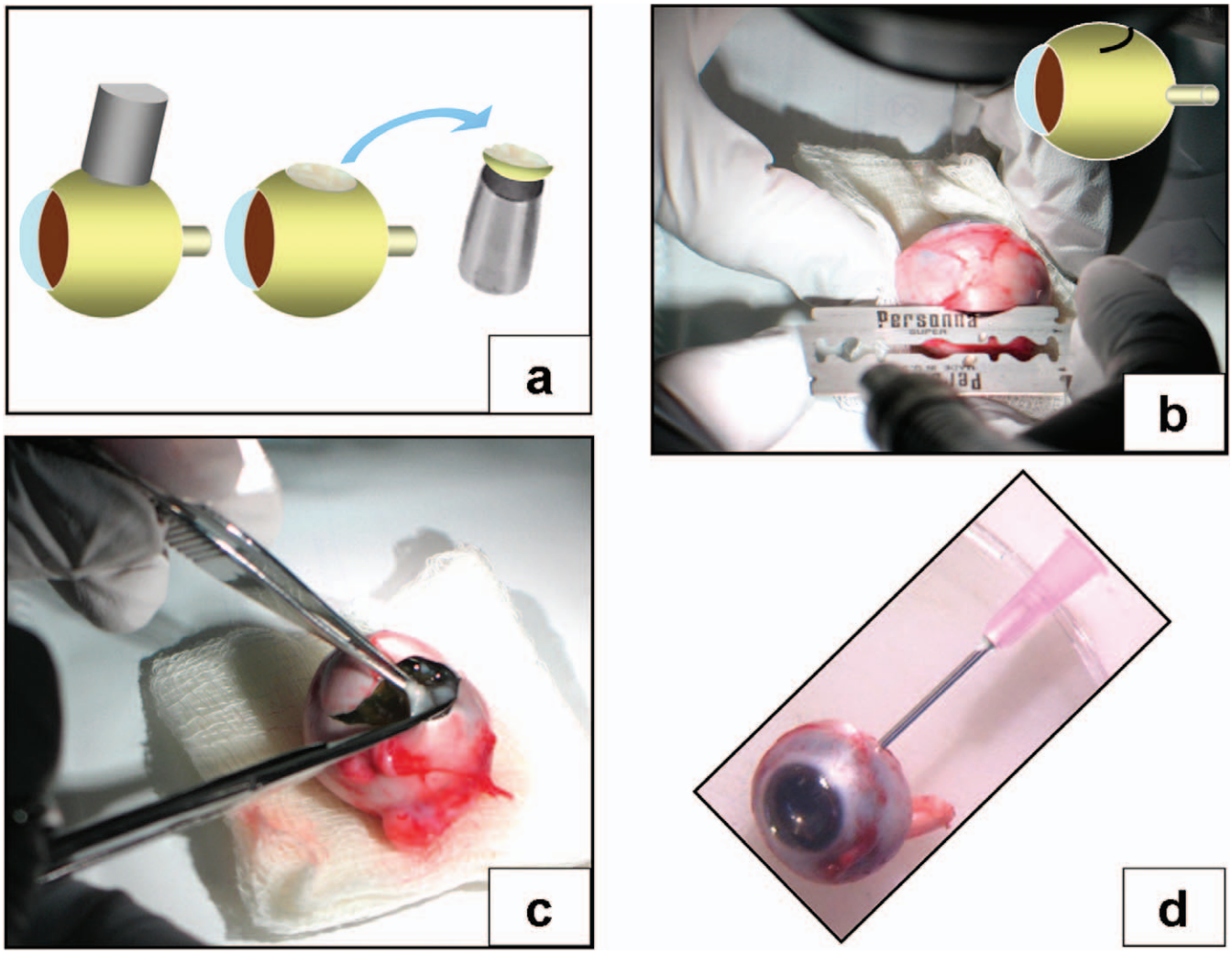

Figure 2. *Techniques for retrieval of fresh tumor. a, Diagram showing use of corneal trephine to excise round disk of full-thickness cornea during corneal transplant surgery. The trephine should be positioned on the sclera overlying or at the edge of the area known to contain the major focus of intraocular tumor (as evidenced by a transillumination shadow or results of prior clinical examination). Trephination and manipulation of the globe should be performed gently to avoid collapsing the eye and inadvertently expulsing the tumor tissue and other intraocular contents. b, The use of stereomicroscopy facilitates evaluation of tumor location and quality of tumor (viable versus necrotic) for retrieval by opening of a scleral window. Using a sharp blade or knife, gently cut a small window in the equatorial sclera overlying the area known to contain the major focus of intraocular tumor (as evidenced by a transillumination shadow or results of prior clinical examination). The incision should be made at a safe distance from the optic nerve (diagram in inset). Note: manipulation and sectioning should be performed gently to avoid collapsing the eye, inadvertently expulsing the tumor tissue and other intraocular contents, and creating artifacts that can interfere with histopathologic interpretation. c, Tumor tissue should be harvested by excising small samples at a time. d, Under sterile conditions, a 22-gauge needle is introduced through the sclera posteriorly to the lens according to a slightly oblique anteroposterior course under visual control (at the site previously identified by transillumination or clinical observation as having the largest amount of tumor). When the needle is situated in the tumor, tumor material is aspirated by connecting a syringe to the needle.*

by connecting a syringe to the needle. In the case of a nonfriable tumor, a few milliliters of culture medium can be introduced, allowing dilution of the tumor material and facilitating aspiration. When the material has been collected, an aliquot may be analyzed to evaluate the tumor cellularity of the aspirate. After tumor harvesting by both techniques, the eye is then placed in sufficient formalin to cover the globe and fixed for at least 48 hours.

Consensus was reached as to the examination of the entire eye microscopically by submitting a total of 4 blocks. One block is the central pupil–optic nerve (PO) section containing the optic nerve, tumor, and anterior chamber structures (Figure 3, a). Two blocks will contain the calottes (remainder of ocular tissue after obtaining the PO) in anterior-posterior segments embedded on edge to examine more choroidal surface (Figure 3, b). The fourth block contains the cross section of the margin of the optic nerve, obtained before opening the eye. The slides of the PO section should contain the optic nerve head, lamina cribrosa, and postlaminar optic nerve in a single plane of section (Figure 3, c).

CONCLUSIONS

The proposed guidelines for definitions of high-risk features and for processing of the eyes with retinoblastoma are based on the previous reports and experiences of experts around the world and were felt to be practical, fea-

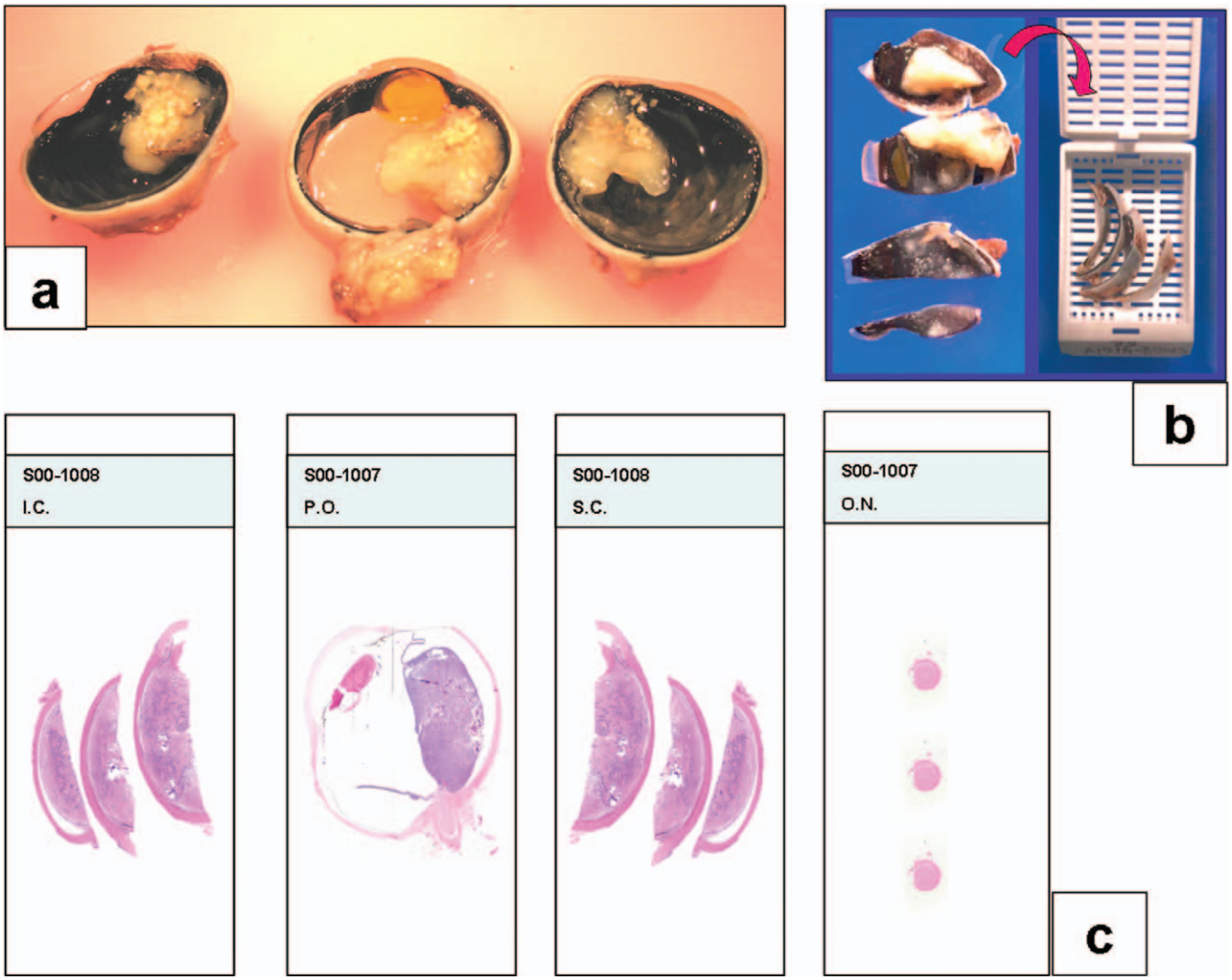

Figure 3. *a, Photograph of the inferior calotte (right), pupil–optic nerve (PO) section (center), and superior calotte with initial cut (left). Notice that most of the tumor is present in the PO section. b, Longitudinal anterior-posterior sections of the calotte are obtained from each calotte and then submitted one cassette per calotte. c, Example of slides for an eye with retinoblastoma. The section labeled P.O. shows a section of the eye with iris at the level of the pupil, lens, tumor and, importantly, optic nerve with lamina cribrosa at the center of the optic nerve for evaluating invasion by tumor. Notice that the slide labeled O.N. has a cross section of the margin of the optic nerve (hematoxylin-eosin, original magnification ×1). I.C. indicates inferior calotte; S.C., superior calotte.*

sible, and objective. Prospective clinical trials and other studies must validate the proposed consensus criteria.

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